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Short communication

Scallop larval survival from erythromycin treated broodstock after conditioning without sediment

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ABSTRACT

Pathogenic bacteria are known to be one of the main factors affecting *Pecten maximus* larval survival in hatcheries. As a result, juvenile production often relies on the use of antibiotics during larval culture. However, limitations of the usage of chemicals such as chloramphenicol in aquaculture have been reinforced due to their negative environmental impact and alternatives are accordingly needed. Thus, the importance of bacterial transfer from oocytes to larvae has been studied here as well as procedures to limit larval mortality in *P. maximus*. In order to reduce bacterial contamination during larval development, we focused on two periods, broodstock conditioning and post-fertilization. The animals were conditioned for 2 months with two erythromycin treatments of 6 days, with (*SA*) or without sandy-bottom (*NSA*). The absence of sediment strongly reduced contamination by *Vibrios* of oocytes (*NSA*: 0.003 ± 0.002 CFU oocyte^{-1} *SA*: 0.57 ± 0.17 CFU oocyte^{-1}) and D-larvae (*NSA*: 0.14 ± 0.05 CFU D-larva^{-1} *SA*: 0.51 ± 0.002 CFU D-larva^{-1}). It also enhanced survival by 52% at 15 days post fertilization, whereas a two day antibiotic treatment of D-larvae did not improve subsequent survival, regardless of broodstock conditioning method. Furthermore, both treatments led to similar fatty acid profiles of oocytes and resulting larvae, suggesting that broodstock conditioning was physiologically similar with or without sediment. This work has shown that gamete contamination could be reduced when sandy-bottom was removed during broodstock conditioning under bacterial control with erythromycin. The present results contribute to a reduced utilization of antimicrobial agents for great scallop larval rearing in controlled condition.

1. Introduction

Compared to oysters or clams (Loosanoff and Davis, 1963; Walne, 1966), the production of Pectinid juveniles for aquaculture, enhancement of populations or sea ranching through reliable hatchery processes is more recent (Dao et al., 1995; Helm et al., 2004). After the first laboratory trials (Gruffydd and Beaumont, 1972), seed supply by hatcheries progressed rapidly. First results of experimental production were encouraging (Buestel et al., 1982), but despite recent progress (Magnesen et al., 2006) juvenile production remains, however, unpredictable due to high variations in larval survival, often associated with massive bacterial infections (Andersen et al., 2011; Devauchelle and Mingant, 1991). To limit bacterial contamination and sustain production of juveniles, the preventive or systematic use of antimicrobial agents seems to be the solution for some Pectinid hatcheries (Le Pennec and Prieur, 1977; Nicolas et al., 1996; Robert et al., 1996; Torkildsen et al., 2005; Uriarte et al., 2001). Larval survival is generally improved but such a practice is unsustainable as it increases the risk of selecting resistant bacteria (Akinbowale et al., 2006; Cordero et al., 2012; Riquelme et al., 1994).

Among heterotrophic bacteria, some *Vibrios* are known to be particularly virulent and have been reported to induce severe and rapid larval mortalities of molluscs (Elston and Leibovitz, 1980; Lambert et al., 1998; Lodeiros et al., 1987). Vertical transfer of bacteria from broodstock to gametes and larvae of different bivalve species has been described in many studies (Avendaño-Herrera et al., 2001; Lodeiros et al., 1987; Prado et al., 2013; Riquelme et al., 1994). In Pecten maximus, gametes and D-larvae contamination could result in a transfer of bacteria from the intestinal loop to the gonad (Beninger et al., 2003; Le Pennec et al., 1992). For the contamination at early life stages, *Vibrio* were shown to be released into seawater rearing by *Argopecten ventricosus* during spawning, inducing bacterial blooms in tanks 24 h post-incubation (Sainz-Hernández and Maeda-Martínez, 2005).

The main objective of the present study was to assess different possibilities to limit the microbial load in early life stages of *P. maximus* larvae. On one hand, the influence of sediment during broodstock conditioning was studied because we hypothesised that early gamete contamination might be induced during broodstock conditioning. Most bivalves do not need sediment to be conditioned in hatchery but

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scallops are generally maintained in tanks with sand or gravel to mimic their natural environment (Utting and Millican, 1997). This sediment could favour bacterial development by trapping microorganisms and becoming a potential gamete infection source. On the other hand, the hypothesis that a short and early treatment, target on broodstock bacterial flora, could noticeably reduce bacterial proliferation and accordingly enhance larval survival was studied. To determine the influence of the presence of sediment during broodstock conditioning on larval quality, fatty acid content and composition have been determined on each oocyte and D-larva sample. Total fatty acid accumulation and composition in bivalve larvae have been largely demonstrated to be a good indicator of larval quality (Delaunay et al., 1992; Gagné et al., 2010; Glencross and Smith, 2001; Le Pennec et al., 1990; Marty et al., 1992; Pernet and Tremblay, 2004; Soudant et al., 1996).

2. Materials and methods

2.1. Broodstock sampling and conditioning

Three–four year-old great *scallops* were collected by diving in the Bay of Brest (Finistère, France) in March 2011 and transferred to the Ifremer experimental hatchery of Argenton (Finistère, France). One hundred and twenty individuals (mean shell height: 108.5 ± 0.7 mm) were distributed in 8 tanks of 300 L (15 adults per tank). According to Mason macroscopic scale (Mason, 1958), modified by Robert et al. (1994), maturation stages ranged from 1 to 3 with 2 as the mean value. Broodstock treatments carried out in duplicate corresponded to tanks equipped with coarse autoclaved sandy-bottom (*SA*) or without any sediment (*NSA*).

During conditioning, the photoperiod was maintained at 15:9 and tanks were supplied with flow-through filtered (1 μm) and UV-treated seawater (50 L h^{-1}). Broodstock were continuously fed with *Pavlova lutheri* (P), *Tisochrysis lutea* (T) and *Skeletonema marinoï* (S), at 1:1:1 equivalent volume and a daily ration of 8×10^9 cell animal day^{-1}. Two antibiotic treatments were applied during a 6 day-period in stagnant seawater with circulation pumps. The first was when animals arrived at the hatchery and the second 4 weeks later. The selected antibiotic (see specific Section 2.3 for details) was used at 8 ppm every two days corresponding to seawater renewal. The rest of the conditioning was realized in a continuous water flow. The temperature was raised at the beginning of the experiment by one degree per day until it reached 15 °C.

2.2. Larval rearing

After 7 weeks, 37% and 40% of the broodstocks spawned after thermal stimulation (+5 °C) for *SA* and *NSA* conditions respectively. Oocytes were fertilized as described in Gruffydd and Beaumont (1970). After 48 h of incubation in 150-L cylinder-cone shape tanks in stagnant seawater at 18 °C, 19.5 ± 7.9% and 41.2 ± 0.2% of *SA* and *NSA* oocytes respectively hatched into D-larvae. Veliger larvae were reared in 5-L beakers in stagnant filtered (1 μm) and UV-treated seawater, renewed each second day, at 18 °C. Each batch of larvae, originated from two previous broodstock treatments (*SA* and *NSA*), was exposed to two different larval treatments: *NT*—without antibiotic and *E*—treated with selected antibiotic during 2 days (from 2 to 4 days post-fertilization: dpf; Fig. 1).

The selected antibiotic (see specific Section 2.3 for details) was added in rearing seawater at a concentration of 8 mg L^{-1} (Robert et al., 1996). Larvae, at an initial density of 10 veligers mL^{-1}, were fed daily with a PTS mixture at 60 cells μL^{-1} (1:1:1 equivalent volume). The experiment ended on day 15, at the beginning of the strict exotrophic larval phase to avoid potential confounding effect with trophic conditions.

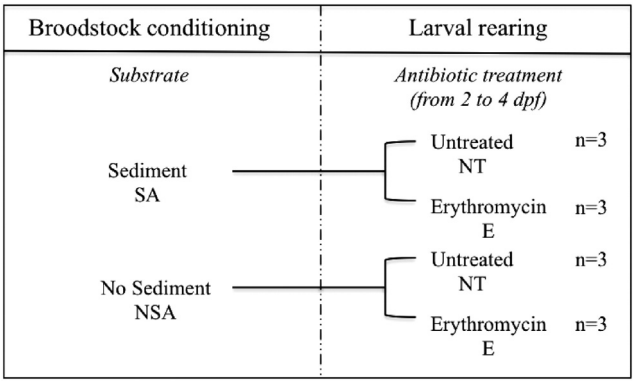

Fig. 1. Schematic of the experimental design: *NT*—without antibiotic and *E*—2 days selected antibiotic treatment.

2.3. Selection of the tested antibiotic

At the onset of the experiment, a portion of gonad and hemolymph of seven scallops were collected and homogenized in sterile seawater (SSW). After 10^{-1} SSW dilution, 100 μL of homogenate was plated onto marine agar. Small disks containing different antibiotics (erythromycin, penicillin, ampicillin, kanamycin, oxytetracycline, tetracycline, streptomycin, ticarcillin, chloramphenicol, flumequin, florfenicol, nalidixic acid, amoxicillin and gentamicin: Sigma-Aldrich, France) were laid in different zones of the culture (Gibson et al., 1998; Prado et al., 2013; Riquelme et al., 1996) and inhibition zones were compared thereafter (Furones, 2001; Mayr-Harting et al., 1972). In our case, erythromycin appeared to be the most efficient antibiotic by presenting the cleanest and largest inhibition zone. This chemical agent has been used for all experiments described in this study.

2.4. Monitoring of bacterial load

Oocytes were sampled for bacterial analysis. During the larval rearing, bacterial concentration was achieved by sampling each larval replicate on a weekly basis. Total flora and *Vibrio* loads were estimated by the plate counting method, using marine agar and Thiosulfate–Citrate–Bile Salts–Sucrose (TCBS) respectively (Azandegbe et al., 2010). They were expressed as Colony Forming Units, CFU $larvae^{-1}$ or $oocytes^{-1}$ for each crushed sample (Thomas Potter in sterile atmosphere before plate inoculation).

2.5. Lipid analysis

Samples of eggs (50,000) and 2 day-old larvae (25,000) were collected onto pre-combusted (450 °C) Whatman GF/C filters, rinsed with 6% ammonium formate and stored at −80 °C in 2 mL of CH_2Cl_2–MeOH (2:1), under nitrogen atmosphere. Lipids were extracted in dichloromethane–methanol (Folch et al., 1957) as described by Parrish (1987) and separated into neutral (including triglycerides, free fatty acids, and sterols) and polar (including mainly phospholipids) fractions as described by Pernet et al (2006). All fatty acid methyl esters (FAMEs) were prepared as described by Lepage and Roy (1984) and analysed in MSMS scan mode on a Polaris Q ion trap coupled to a Trace GC (Thermo Finnigan, Mississauga, ON, CA), as described by Gendron et al. (2013).

2.6. Statistical analysis

We used Permutational Analyses of Variances (PERMANOVA-PRIMER-E 6.0 PERMANOVA plus; PRIMER-E Ltd, Plymouth, UK) because of non-normal distribution of our larval rearing databases. This